

CORRELATION BETWEEN NEUROSECRETORY CHANGES AND MATURATION
OF THE REPRODUCTIVE TRACT OF *ARION ATER*
(STYLOMMATOPHORA: ARIONIDAE)

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ABSTRACT

The nervous systems of *Arion ater* (Linnaeus) from animals at various known stages in the reproductive maturation cycle were examined for neurosecretory material by staining serial sections with Paraldehyde-fuchsin. Patterns of neurosecretory material in nerve cells and tracts of such material in the axons were mapped for the 3 main ganglion masses of the central nervous system (the supra-oesophageal, pleuro-parieto-visceral and pedal ganglion masses) and were obtained from the buccal ganglia and the optic tentacles. The main correlation shown between the maturation of the reproductive tract and neurosecretion occurred at a "critical point", the onset of maturation of the female reproductive glands. Certain small cells in the pleuro-parieto-visceral ganglion mass showed neurosecretory activity at this stage only. This stage is termed "critical", because experimental adverse conditions grossly retarded maturation if applied before this stage, but did not affect it much if applied after that stage had been reached. A possible control system for the maturation of the reproductive cycle is postulated from these results.

INTRODUCTION

Several workers have recently described various aspects of maturation of the pulmonate reproductive tract and suggested possible controlling factors. Laviolette (1954) described the role of the gonad in the maturation of the reproductive tract of the Arionidae, suggesting a hormonal system. Herlant-Meewis & van Mol (1959) suggested a relationship between neurosecretory activity and reproduction in 2 species of *Arion*, and Pelluet & Lane (1961) described the control of gamete maturation by brain and eyestalk substances in *Arion* species. Recently the present author (Smith, 1966a) described the

various maturation stages of the reproductive tract of *Arion ater* (Linnaeus) and found a close correlation between maturation and the season. The present study, based on the same specimens, was undertaken in order to ascertain the correlation, if any, between neurosecretory activity and maturation of the reproductive cycles. It should be noted that the work was carried out in England, and that months mentioned in connection with changes in the reproductive tract should be correlated with northern hemisphere seasons. As a foundation for the investigation of the patterns of neurosecretion, the anatomy of the central nervous system of *Arion ater* was described in detail (Smith,

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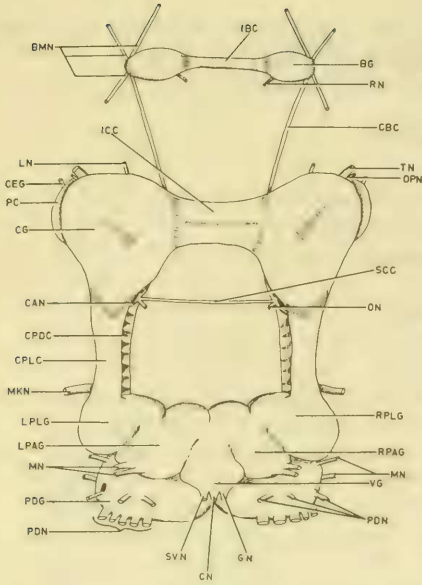


FIG. 1. A diagrammatic posterior view of the central nervous system showing the general anatomy and the origins of the nerves.

KEY TO ABBREVIATIONS

- ANT anterior
- AX axon
- BG buccal ganglion
- BMN buccal mass nerve
- CAN cerebral anastomosis
- CBC cerebro-buccal connective
- CEG cephalic gland
- CG cerebral ganglion
- CN cardiac nerve
- CPDC cerebro-pedal connective
- CPLC cerebro-pleural connective
- DCG dorsal cell group
- GN genital nerve
- IBC inter-buccal commissure
- ICC inter-cerebral commissure
- LN lip nerve
- LPAG left parietal ganglion
- LPLG left pleural ganglion
- MKN mantle & kidney nerve
- MN mantle nerve
- MSC mesocerebrum
- MTC metacerebrum
- NS neurosecretory drops
- OC otocyst
- OCN otocyst nerve
- ON oesophageal nerve
- OPN optic nerve
- PC procerebrum
- PCG posterior cell group
- PDG pedal ganglion
- PDN pedal nerve
- PLPDC pleuro-pedal-connection
- PPVG pleuro-parieto-visceral ganglion mass
- RN radular nerve
- RPAG right parietal ganglion
- RPLG right pleural ganglion
- SCC subcerebral commissure
- SVN stomach and visceral nerve
- T tract of secretion
- TN tentacular nerve
- VCG ventral cell group
- VG visceral ganglion
- VN visceral nerve
- VLCG ventro-lateral cell group
- VMCG ventro-mesial cell group

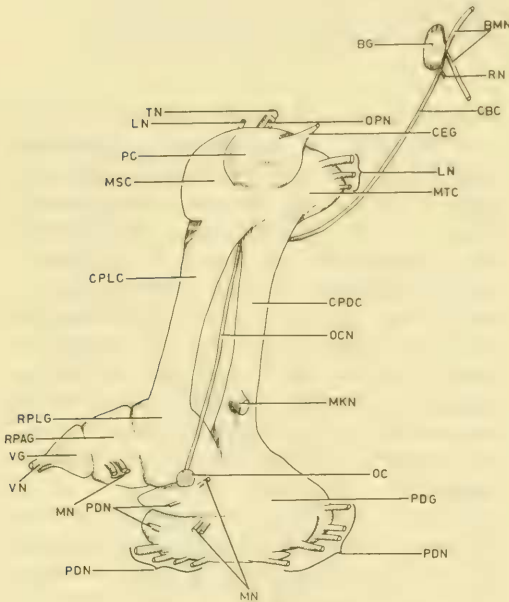


FIG. 2. A diagrammatic lateral view of the central nervous system from the right side showing the general anatomy and the origins of nerves.

1966b). For the purpose of this investigation, neurosecretory material is defined as paraldehyde-fuchsin positive material. The validity of this definition has been discussed elsewhere (Smith, 1966b).

MATERIALS AND METHODS

A total of 65 central nervous systems and optic tentacles from animals used in the determination of the maturation stages of the reproductive tract of *Arion ater* (Smith, 1966a) have been examined: the maturation stage of each individual is thus precisely known. These slugs comprized a natural population as well as individuals from controlled environment experiments. Since the techniques used were time consuming, the investigation was limited to a few specimens (2-4) representative of each maturation stage of the hermaphrodite gland, from each of the various groups.

The nervous systems were fixed in Susa for 6 hours or in Elftman-Dichromate-Sublimate for 3 days (Elftman, 1957). They were then transferred to Cellosolve, embedded in Ester Wax and serial sections were cut at 10μ . These were stained in Paraldehyde-fuchsin using a slight modification of the technique of Gabe (1953) (Smith, 1966b). The distribution of positive staining material in the nerve cell bodies and nerve fibres was then mapped at each hermaphrodite gland stage (see below) and the maps compared. Cell sizes were measured by means of a micrometer eye piece.

GENERAL ANATOMY OF THE CENTRAL NERVOUS SYSTEM

The detailed anatomy of the central nervous system of *Arion ater* has been described elsewhere (Smith, 1966b). It is sufficient here to give a brief outline. The pulmonate central nervous system consists of a highly complex circum-oesophageal ring formed by the amalgamation of the 9 primitive pulmonate ganglia (Bargmann, 1930). For con-

venience of description it is easier to divide the nerve ring in *Arion* into 3 large ganglion masses (Figs. 1 and 2):

1. The supra-oesophageal ganglion mass,
2. the pleuro-parieto-visceral ganglion mass,
3. the pedal ganglion mass.

Although the grooves on the surface of the ganglion masses do not necessarily indicate the positions of fusion of the original constituent ganglia, it is convenient to name the parts suggested by these grooves as if they were in fact the constituent ganglia.

PATTERNS OF NEUROSECRETION AT DIFFERENT STAGES OF MATURATION OF THE HERMAPHRODITE GLAND

Wherever possible animals were chosen from collections covering as wide a range of dates as possible in order to attempt to differentiate seasonal from maturation changes. Each part of the nervous system is considered separately, descriptions of neurosecretion being given with special reference to the states of activity of the secretory cells and to the presence and routes of any tracts of neurosecretion. Diagrams of each of the ganglion masses are given showing the distribution of the neurosecretory tracts and their relationships to the cell groups. The tracts are referred to in the text by numbers which correspond to those given in the diagrams.

The reproductive stages of the hermaphrodite gland, described in detail in an earlier paper (Smith, 1966a) are briefly summarized as follows:

STAGE

- A: Spermatogonia and spermatocyte
- B: Early Spermatid
- C: Late Spermatid
- D: Early Spermatozoa
- E: Mid Spermatozoa
- F: Late Spermatozoa
- G: Early Oocyte

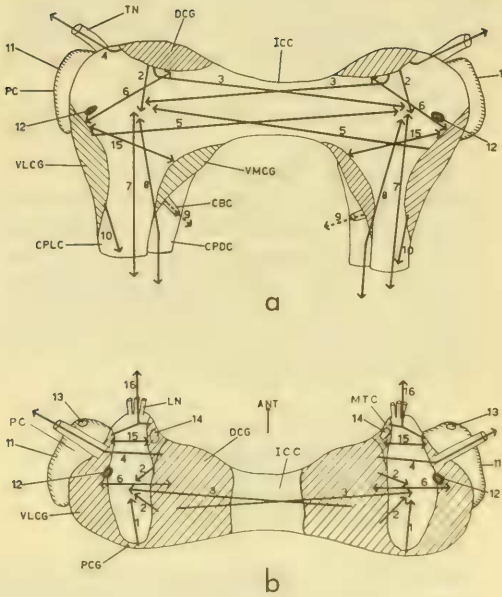


FIG. 3. Diagrams of the posterior (a) and dorsal (b) views of the supra-oesophageal ganglion mass showing the distribution of neurosecretory cells and tracts. For an explanation of the numbers see text.

H: Late Oocyte
I: Atrophy

The titles of these stages denote the predominant component in sections of the hermaphrodite gland. Where 2 or more stages go under the same gametogenesis designation but can be distinguished histologically, they are distinguished temporally. The main significant period in the maturation of the reproductive tract occurs during Stage E of the hermaphrodite gland, when mature sperm commence to leave the gland and copulation occurs. At that time all the female reproductive glands also differentiate and start to mature. The significance of this stage will be discussed in more detail later.

Supra-oesophageal Ganglion Mass

The pattern of neurosecretion in this ganglion mass is highly complex, but seems to be largely independent of the

state of the maturation of the reproductive system. The distribution of the neurosecretory cells and tracts, or paths, of secretion that are present for all or part of the maturation cycle is summarised in Fig. 3.

The posterior cell group (PCG, Fig. 3b) produces secretion which forms well defined tracts of neuro-secretory material (1) leading into the central fibrous ganglionic mass, where their course can no longer be followed. The dorsal group of cells (DCG, Fig. 3a, b; Fig. 4) produces a large amount of secretion which is passed out in tracts (2, Figs. 3a, b; T, Fig. 4) which also enter the central fibrous mass. Most of these tracts are short and connect with the mass of the same ganglion; some, however, pass through the inter-cerebral commissure (ICC) to the mass of the other cerebral ganglion (3). The cells in the dorsal group are usually full of secretion. A small tract of secretion arises from the small cells in the anterior-lateral part of the dorsal cell group and passes to the tentacular and optic nerves (4, Fig. 3a, b). A group of small cells (10-20 μ long) in the middle of the ventro-lateral cell group (VLCG) also produce secretion which passes along a tract to the fibrous mass of the opposite cerebral ganglion through the inter-cerebral commissure (5, Fig. 3a). Also associated with this group of cells is a well defined tract connecting it to the dorsal cell group (6). Whether the secretion in this tract originates in only one or both of these centres is not certain.

Tracts are seen in the cerebro-pleural (CPLC; 7, Fig. 3b) cerebro-pedal (CPDC; 8) and possibly in the cerebro-buccal connectives (BC; 9). These tracts connect the fibrous masses of the ganglia, but it is not certain in which direction the secretion is being transmitted. As much more secretion is present in the cerebral ganglia than in the others, it is considered possible that the secretion is passing out from the cerebral ganglia. There is also a small tract (10, Fig. 3a)



FIG. 4. Tracts of secretion (2, of Fig. 3) leading from the dorsal cell group of the cerebral ganglion. Susa, Paraldehyde-fuchsin.

from some small and medium sized cells ($15-35\mu$) in the ventro-lateral cell group (VLCG) down the cerebro-pleural connective (CPLC).

The procerebrum (PC) is largely empty of secretion. There is however a single layer of positive cells ($15-20\mu$ long) in some areas around the periphery of the lobe (11) towards its anterior end. At the ventral side of the procerebrum is a single large cell (12), $30-60\mu$ long, which is always full of large droplets of secretion, some $1-1.5\mu$ in diameter. The cephalic gland (CEG, Figs. 1,2) and vesicle at the anterior end of the procerebrum also usually have a few drops of secretory material associated with them (13).

The giant cell (14) in the mesial cell group of the metacerebrum (MTC) usually contains secretory droplets and the small cells ($10-20\mu$) surrounding it also have positive material. There is a tract (15) connecting these cells (VMCG, Fig. 3a) and cells of a similar size in the lateral cell group (VLCG). Finally, from the cells at the anterior end of the mesial and lateral groups originate tracts (16) which pass down the lip (LN) and inferior tentacular nerves.

The pattern of neurosecretory activity in these cells and tracts is largely similar throughout the year. There are, however, some differences in the distribution of neurosecretion related either directly or indirectly to the reproductive cycle or the season of the year or both.

In the spermatocyte stage (A) very early in the season there is little secretion present. Most of the tracts and positive cell groups, however, show some activity except for 3 areas; positive material is absent from around the vesicle (13) associated with the cephalic gland, from the lateral cells (VLCG) near the cerebro-pleural connective and from the tract (10) from these down the connective. One animal in this stage was from the end of the season (November 3rd) and was probably only 6-8 weeks old. This animal showed more secretion than animals at the same stage at the beginning of the year and the only deficiency it showed was the lack of secretion in the lateral cells and of the tract (10) originating from them.

More secretion is present at the subsequent early spermatid stage (B) but there is still no secretion around the vesicle (13) or in the lateral cells (VLCG).

In the late spermatid stage (C) still more secretion is present. The vesicle is much bigger and there are small drops of positive material surrounding it, as well as in some of the cells in the cephalic gland. In this stage the lateral cells also begin to secrete and tract (10) arising from them is seen.

In the early spermatozoa stage (D) there seemed to be less secretion than in the previous stage, but as only 2 animals were available, this result is uncertain. The vesicle is very large and seems to be filled with some refractile, paraldehyde-fuchsin negative material.

The mid-spermatozoa stage (E) has approximately the same amount of secretion as the late spermatid stage (C) but the distribution seems to be slightly different. There is slightly more secretion at the posterior end of

the ganglion and slightly less at the anterior end. The vesicle (13) had partially collapsed in 4 out of 5 animals, though there was still positive material surrounding it. In 2 animals in this stage, positive tracts (9) were seen leading from the fibrous mass to the buccal connectives (CBC, Fig. 3a).

The late spermatozoa stage (F) has much more secretion than the previous stages. This is probably due to a marked increase in activity in the dorsal cell group (DCG). The tracts arising from it show large drops of secretion along their length. The vesicle is smaller but the associated cephalic gland is still large.

In the early and late oocyte stages (G, H) the amount of secretion is considerably less than in the preceding late spermatozoa stage (F). Only 2 animals were found in the atrophy stage (I), one in May which had probably overwintered and was at a very late stage, and the other in December. Both of these animals showed a small reduction in the quantity of secretion compared with the oocyte stages. They did, however, show all the tracts and had much more secretion than was found in the spermatozoocyte (A) or the spermatid (B, C) stages. Thus the animal in the atrophy stage in May had a greater amount of secretion in the supra-oesophageal ganglion mass than the animals in the early stages of maturation at the same time.

Pleuro-parieto-visceral Ganglion Mass

This ganglion mass also shows a complex pattern of neurosecretion throughout the year. The changes from one reproductive stage to another are greater than in the cerebral ganglia and seem to have some relationship to the reproductive stages.

The distribution of the neurosecretory cells and the tracts of secretion are summarized in Fig. 5. Tracts of secretion (17) from the cerebro-pleural connective (CPLC) enter (or leave) the fibrous mass of the pleural ganglia (LPLG). Tracts leave this mass (18)

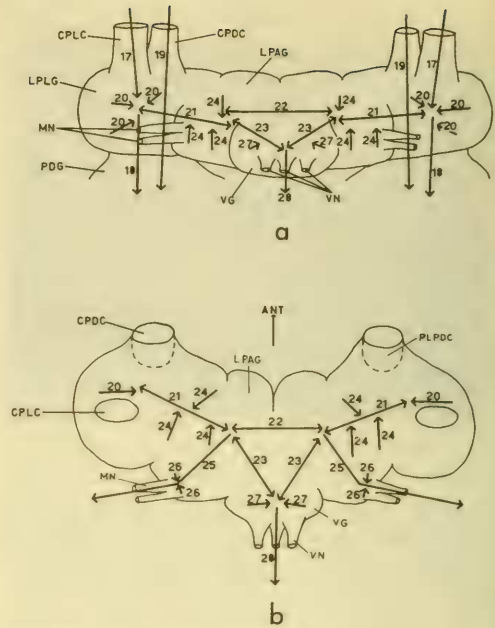


FIG. 5. Diagrams of the posterior (a) and dorsal (b) views of the pleuro-parieto-visceral ganglion mass showing the distribution of neurosecretory cells and tracts. For an explanation of the numbers see text.

and apparently pass through the pedal ganglion (PDG), while other tracts (19), coming down the cerebro-pedal connective (CPDC) pass through the anterior part of the ganglion to the pedal ganglion. At certain times of the year many small cells (15-35 μ long) surrounding the fibrous mass become filled with secretory droplets and the tracts of secretion from these cells (20) enter the fibrous mass. These cells are found mainly in groups surrounding the pleuro-pedal connections (PLPDC), the area of fusion of the pleural and parietal ganglia and in cells lateral to the central fibrous mass in the pleural ganglia.

Various neurosecretion tracts connect the ganglia of this complex: the pleural-parietal (21), inter-parietal (22), parietal-visceral (23) tracts. The actual course of the various nerve axons are impossible to trace but there is evidently a great deal of interchange of neurosecretory material between all

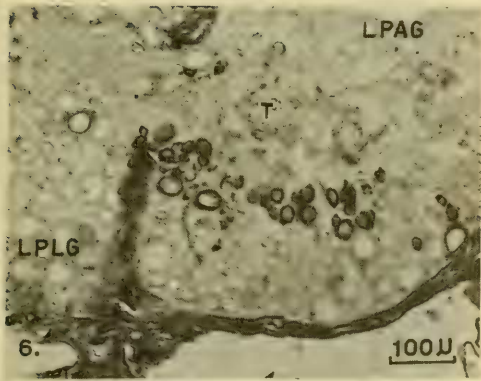


FIG. 6. A group of small cells in the ventral part of the parietal ganglion and around the parieto-pleural connection, full of secretion. Susa, Paraldehyde-fuchsin.

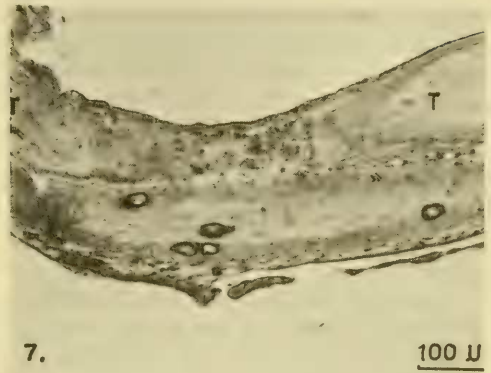


FIG. 7. The 2 mantle nerves of the parietal ganglion with small cells containing neurosecretory material and secretory tracts. Susa, Paraldehyde-fuchsin.

parts of this ganglion mass. The cells in the parietal ganglia are very active, neurosecretory material of varying quantity being present at all times of the year. Giant cells can be observed in all stages of secretion and storage, especially in the posterior and ventral parts of the ganglia. In certain periods the small cells ($15-35\mu$) surrounding the fibrous mass are very active, especially in the ventral parts of the ganglia and around the pleuro-parietal connection (Fig. 6). These cells give rise to tracts (24, Fig. 5a, b) which run into the fibrous mass. Large tracts (25, Fig. 5b) from the parietal ganglia run down the large mantle nerves (MN). At the points of origin of these nerves there occur a number of small cells which also become very active at certain times of the year and give rise to tracts apparently running into the tracts to the mantle nerves (26). Small positive cells (20μ) are also seen along the length of the mantle nerves near their origins from the ganglion (Fig. 7) in a few animals at certain times of the year.

The cells in the visceral ganglion (VG) usually show some secretory activity throughout the year. It is however very slight. The secretions of the giant cells

run in tracts to join the parietal-visceral tract in the fibrous mass (27). From this fibrous mass, tracts (28) run down all 3 of the visceral nerves.

As has been previously stated the amounts and distribution of neurosecretory material vary for different stages of reproductive maturation, and there seems to be some sort of relationship between the 2 as will be discussed later (p 295).

The spermatocyte stage (A) has very little secretion, but all the main tracts between the ganglia and the ganglion mass can be found. Most giant cells contain a few secretory droplets and 1-2 large cells ($50-80\mu$) in the posterior part of the parietal ganglia contain very many large drops of secretion. There are however, no small, positive cells surrounding the fibrous mass in the parietal ganglia, and no secretion at all in any of the cells of the pleural ganglia. This is also true of the animal collected in the very young stage, on November 3rd, although in the "frothy" cells, in the anterior lobe of the left parietal ganglion, there were many droplets. The droplets, however, occur in the cytoplasm of the cell and not in, or associated with, the large vacuoles.

The same distribution of neurosecretory material is seen in the early and late spermatid stages (B,C) though an increase in the amount of secretion in both the cells and tracts can be observed. In addition, in the late spermatid (C) and early spermatozoa (D) stages, a few cells surrounding the fibrous mass in the pleural ganglion are seen to contain a small amount of secretory material.

A major change is noticed in the distribution and amount of secretory material in this ganglion mass in the mid-spermatozoa stage (E), the stage when the first signs of maturation of the female part of the reproductive tract is observed. Much more secretion is found in all the tracts. Large amounts of neurosecretory material appear within the very small cells that occur in groups around the fibrous mass of the parietal ganglia, mainly in their ventral part, around the pleuro-parietal connection (Fig. 6) and around the origin of the mantle nerves. The tracts leading from these cells into the fibrous mass are filled with large amounts of secretion. In addition many small secretory cells occur in the lateral cell group of the pleural ganglia and around the pleuro-parietal and pleuro-pedal connections. These cells give rise to tracts which run into the pleural fibrous mass. The amount of secretion in the tracts running from the pleural into the pedal ganglia is also greatly increased.

The late spermatozoa stage (F) shows a further increase in secretory material, again with a slight increase in the number of small cells secreting. It seems to be the stage of maximum secretion, since the early and late oocyte stages (G, H) show a decrease in the amount of secretion and also a sharp decrease in the numbers of these small secreting cells. The greatest decrease is evident in the pleural ganglia, where, by the end of the late oocyte stage, only 1-2 small cells with secretory droplets are present. The larger secretory cells show little change in neurosecretory activity

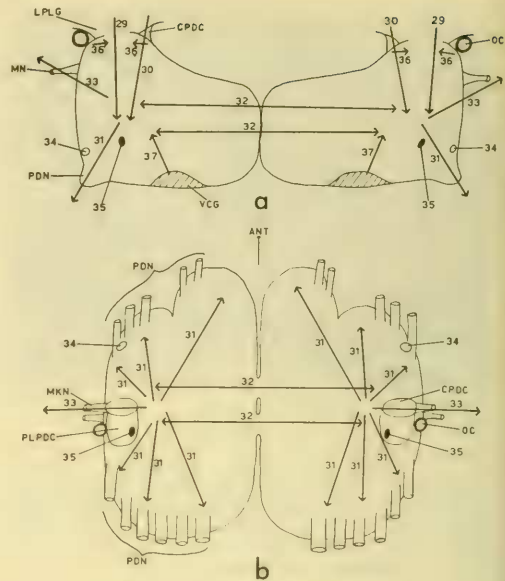


FIG. 8. Diagrams of the posterior (a) and dorsal (b) views of the pedal ganglion mass showing the distribution of neurosecretory cells and tracts. For an explanation of the numbers see text.

over this period. Both the animals in the atrophy stage (I) had a few of these small cells containing secretory material. In both cases the amount of secretion was greater than that in the young stages. In the animal collected in May, which had reproduced and overwintered, there was still a large amount of secretion; one peculiar feature was the presence of many droplets of secretion between the very large vacuoles in the "frothy" cells of the left parietal ganglion.

Pedal Ganglion Mass

This ganglion mass has very little neurosecretory activity and there does not seem to be any correlation between the activity it does show and the reproductive maturation cycle. The distribution of neurosecretory cells and tracts are summarized in Fig. 8.

Large tracts of secretion enter the fibrous mass from the pleural ganglia

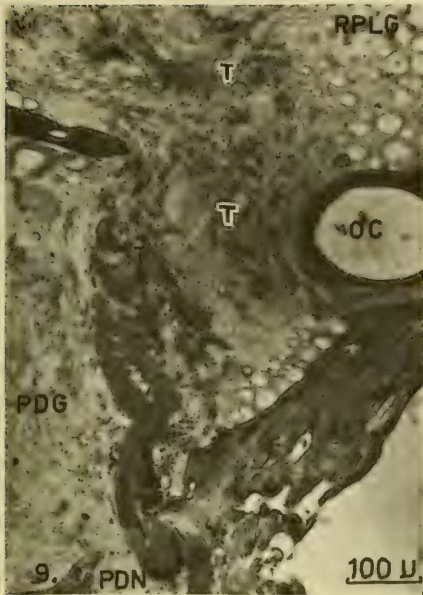


FIG. 9. Tracts of secretion (31) from the pleural ganglion through the pedal ganglion and into the pedal nerve. Susa, Paraldehydrofuchsin.

(29) and cerebro-pedal connectives (30). From this fibrous mass tracts (31) run down the pedal nerves (PDN, Fig. 8a, b; Fig. 9). Besides these, large tracts (32) run between the 2 pedal ganglia through the 2 inter-pedal commissures. Tracts (33) also lead from the fibrous mass down the lateral mantle nerves (MN, Fig. 8a; MKN, Fig. 8b). Very few cells in the pedal ganglia produce much secretion although a few secretory droplets can be found in practically all the cells. This is particularly true of the few giant cells in the anterior half of the ganglia where some positive droplets are nearly always found. One giant cell in particular (34), 80-120 μ in length, is always full of large droplets and is found in all the nerve rings examined. It is situated in the anterior third of the ganglion in the group of cells lateral to the central fibrous mass and usually just above the origin of one of the pedal nerves. Another cell (35) that is always full of large drops of secretion is a

medium sized cell (30-60 μ long) situated approximately half way along the length of the ganglion directly beneath the pleuro-pedal connection (PLPDC) and embedded in the fibrous mass immediately dorsal to the origin of one of the pedal nerves. There are a few secretory cells which only occur at certain times of the year. A few small cells (15-35 μ) occur around the pleuro-pedal connection (36) and similar cells (19 μ -20 μ) in the ventral cell group (VCG) give rise to tracts (37) which run into the fibrous mass.

The distribution of neurosecretion in the pedal ganglia is largely the same throughout the year. The amount of the secretion in the tracts changes according to the amount passed in from the pleural and cerebral ganglia, being a maximum in the late spermatozoa stage (F). The only exceptions to this are the 2 groups of small secretory cells which are empty of secretion until the late spermatozoa stage, when they commence secreting and continue for the rest of the reproductive cycle.

Buccal Ganglia

Contrary to the results reported by Hekstra & Lever (1960) for *Lymnaea stagnalis*, the cells of the buccal ganglia of *Arion ater* show a great deal of neurosecretory activity. Nearly all the cells contain some neurosecretory material and most cells usually contain considerable numbers of large positive droplets (Fig. 10). The lateral giant cells in particular show intense secretory activity. Another feature found in all the buccal ganglia examined is the presence of a few small cells (10-25 μ long) full of large secretory droplets around the origin of the radular nerve. Large amounts of secretion are found in tracts within the fibrous mass of the ganglion, in all nerves and connectives arising from the ganglia, and in the inter-buccal commissure.

This distribution of neurosecretory material is seen throughout the year with very little change in the amount of

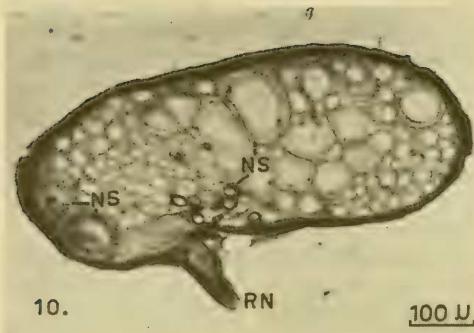


FIG. 10. Buccal ganglion showing neurosecretory material present in most of the cells. Susa, Paraldehyde-fuchsin.

secretion present. There seems, therefore, to be no correlation between the secretion produced in the buccal ganglia and reproductive maturation.

Optic Tentacles

Lane (1962) described the anatomy of the optic tentacles of a number of stylomatophorans and reported neurosecretion (paraldehyde-fuchsin positive material) in 'collar and lateral cells' in the optic tentacle. In the present investigation the optic tentacles of *Arion ater* were examined, collar and lateral cells as described by Lane were recognised and paraldehyde-fuchsin positive material was found in both types. Tracts of secretion were also found in the digitated ganglion and the tentacular nerve associated with it in the tip of the optic tentacle. These tracts, however, have not been connected with the collar and lateral cells. Paraldehyde-fuchsin positive material also occurs in considerable quantities at the ends of the finger-like processes of the digitated ganglion in or around the sensory endings in the tentacular epidermis. It also occurs in considerable quantities in the retinal cells of the eye. Pelluet & Lane (1961) postulated that neurosecretion is only produced in the optic tentacle

at certain periods during the reproductive cycle. This is at variance with the present investigation as tracts containing approximately the same amounts of secretion are observed in all stages of the maturation cycle. There also seems to be a large variation in the positive material in the collar and lateral cells within a single maturation stage.

EFFECT OF ENVIRONMENTAL FACTORS ON NEUROSECRETION AND REPRODUCTIVE MATURATION

In a previous paper it was shown (Smith, 1966a) that, although it was possible to advance or retard, with respect to the natural population, the maturation of the reproductive tract of animals by controlling one or more factors of their environment, it was not possible to alter the relationship between the hermaphrodite gland stage and the stage of maturation of the remainder of the tract. This means that a particular hermaphrodite gland maturation stage always corresponds to the same particular maturation stage of the remainder of the reproductive tract. As part of the present investigation, the pattern of neurosecretion was also mapped for the animals subjected to these environmental factors. In all cases exactly the same relationship between the neurosecretory activity and the hermaphrodite gland stage was observed as that already described above from animals of the natural population.

DISCUSSION

In the interpretation of the patterns of neurosecretion it is necessary to consider exactly what a large concentration of stainable material implies. One explanation might be that secretion is only seen in large quantities when it is being stored in the cell producing it, or in the axon, rather than being transported to the blood or target organ. If this were the case, a cell that was actively

secreting and constantly passing its secretion out down the axon would show little or no stainable material, as the material would be passed out as fast as it was being formed. The tracts from an actively secreting group of cells would then show little or no reaction, while the large prominent tracts would indicate material that was being stored and not sent out into the body. Because the greatest quantity of secretion is found at what can be assumed to be the animals' most active season physiologically (at the height of reproductive activity), and assuming that the positive staining material is in fact a neurohumour or a hormone, it follows that the above explanation would imply that the majority of substances produced by these cells are inhibitory in function; an unlikely explanation, that is probably incorrect.

An alternative explanation, probably nearer the truth, is that the amounts of secretion seen at any one time give an accurate guide as to the production and secretion rates of the cells and of the quantity of secretion actually passing through the axon. By this explanation the presence of secretion in a tract or cell implies active secretion of a substance into the blood or its passage down the axon to the target organ. It is also implied that the secretion present must be active at that time.

Although this second explanation seems to account for most of the secretions seen, it is possible that some of the cells may be storing the secretion. Krause (1960) has described storage cells, 'Sackzellen', in various parts of the central nervous system of *Helix pomatia*; while the author has been unable to demonstrate their presence in *Arion ater*, it is possible that the cells containing the large drops of secretion, that were found in the visceral and parietal ganglia, could be storage cells.

As described in a previous paper (Smith, 1966a), there appears to be one major period of change in the maturation of the reproductive system of *Arion ater* to which much of the activity of

the reproductive tract can be related. This critical period occurs during the mid- and late spermatozoa stage (E,F) of the hermaphrodite gland and marks the onset of maturation of all female accessory glands and also the beginning of copulation. In view of the difficulties of interpreting the results obtained by staining for neurosecretion with paraldehyde-fuchsin, it is of great interest to find that the only major change in the pattern of neurosecretion, that can be associated with reproduction, also occurs in the mid- and late spermatozoa stages. At that time large amounts of secretory material suddenly appear in various groups of small cells in the pleuro-parieto-visceral ganglion mass. This secretion is only found in animals in which the female glands have started to mature. It rises to a maximum by the late spermatozoa stage (F), when copulation occurs, and then decreases rapidly in quantity until very little is present by the late oocyte stage (H). The sudden appearance of large amounts of secretory material at this stage loosely agrees with Pelluet & Lane's (1961) theory that there is a brain hormone controlling egg production or the female stage. The relationship between the presence of secretion in these small cells and the maturation of the female glands is always found, even when the onset of the female stage has been artificially advanced.

As the secretion from the small cells in the pleuro-parieto-visceral ganglion mass only occurs for a fairly short period, at the time of the major change in the reproductive tract it would seem reasonable to consider it as a trigger substance which initiates the change rather than maintains it. The actual mechanism of action is not known and could only be elucidated by an experimental approach. It could have a direct action on the reproductive tract as a whole, or an indirect action on the secretory centres of the hermaphrodite gland, which then might secrete an active substance causing the enlargement

of the female glands, as suggested by Laviolette (1954).

There are many more groups of neurosecretory cells and tracts in the nervous system besides those mentioned above. Their secretion seems to vary with the season, in quantity, though not in distribution, and is probably concerned mainly with general metabolic processes. Thus when the pattern of neurosecretion is examined for each hermaphrodite gland stage it is seen that the general level of secretion is also at a maximum at mid- and late spermatozoa stage. Such a situation would, however, be compatible with the intense reproductive activity and possibly with the intensification of other metabolic processes occurring at that time. It is interesting to note here that van Mol (1961) describes the secretion of the cephalic gland in *Arion rufus* as attaining maximum secretion at the corresponding season. This fact was also noted for *Arion ater* in the present investigation; but, since the collapsing and emptying of the cephalic gland appears to be fairly closely correlated with the late spermatozoa stage, the function of the secretion of the gland may be more closely connected to reproductive activity than is suggested by van Mol. However, as the colloidal secretion of the gland is not stained by paraldehyde-fuchsin it is not known whether it is discharged before, or at the time of gland collapse. For this reason no firm conclusion can be drawn as to the role, if any, played by the cephalic gland in reproductive maturation.

No specific relationships could be established between neurosecretion and the onset of differentiation of the male glands and it is possible that this onset is directly connected with general growth and metabolism. Though tracts of positive material were found in the tentacular nerve and in the collar and lateral cells of the optic tentacle, a connection between these cells and the tracts, as suggested by Lane (1962) could not be found; nor was there a relationship established between this positive

material and the reproductive maturation as suggested by Pelluet & Lane (1961) for *Arion subfuscus* and *Arion ater*. The results of this investigation do not entirely agree with Pelluet & Lane's hypothesis of a dual neurosecretory control of reproduction. They postulated the production of a tentacular "hormone" which suppresses egg production while stimulating sperm production and a brain "hormone" which stimulates egg production. The theory that there are 2 substances involved is not incompatible with the present work. The brain "hormone" postulated by them could be equivalent to the secretion of the small cells described above. It is with the mechanism of action of these "hormones" suggested by these authors that this investigation is in disagreement. Pelluet & Lane (1961) suggest that both these "hormones" are produced continually, but that in the young animals the tentacular substance dominates over the brain substance, while after maturity the relationship is reversed. This part of their hypothesis does not agree with the present findings: if it is assumed that their 'brain hormone' is equivalent to the secretion from the small cells of the pleuro-parieto-visceral ganglion mass (see p 295) described in this investigation, then that hormone is not secreted continually but only over a short critical period.

As already mentioned, the stage of maturation in which the female glands start to mature coincides with a "critical point" in the maturation cycle (Smith, 1966a). When the animals were subjected to grossly adverse conditions, such as extremely low temperature, before this critical point was reached, then the maturation cycle was grossly affected; whereas the maturation cycle was affected very little if the critical point had been reached before the adverse conditions were applied.

From all these results it is possible to postulate a control mechanism for reproductive maturation in *Arion ater*. At the beginning of the year, in favour-

able environmental conditions, there is a growth of the animal and of the male part of the reproductive system. It is possible that the maturation of the male glands is influenced by secretion from the hermaphrodite gland. At the mid-spermatozoa stage of the hermaphrodite gland a secretion is possibly produced by the gland or tract, which stimulates the production of a "trigger" substance by the small cells in the pleuro-parieto-visceral ganglion mass. This substance triggers off the changes occurring subsequent to the "critical point". It is possible that this "trigger" substance acts on the hermaphrodite gland which then controls the maturation of the remainder of the tract (Lavolette, 1954).

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RESUMEN

CORRELACION ENTRE CAMBIOS NEUROSECRETORES Y MADUREZ DEL TRACTO REPRODUCTOR DE ARION ATER

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Los sistemas nerviosos en varios individuos de *Arion ater* (L.) de diferentes estados de maduración conocidos en el ciclo reproductivo, se examinaron para localizar

muestras neurosecretoras, tífindo series seccionadas con paradelhido-fucsina. Muestras neurosecretoras en células nerviosas y ductos de tales materiales en los axones, fueron cartografiadas de las 3 masas principales de ganglios del sistema nervioso central (supraesofágico, pleuro-parieto-visceral y pedales), y de los ganglios bucales y tentáculos ópticos. La correlación principal entre maduración del ducto reproductor y neurosecreción, ocurrió en el "punto crítico", el comienzo de madurez de las glándulas reproductoras femeninas. Ciertas pequeñas células en la masa ganglionar pleuro-parieto-visceral, mostraron solamente actividad neurosecretora. Este estado es denominado "crítico" porque condiciones experimentales adversas retardaron groseramente la maduración cuando se aplicaron previamente a tal estado, pero sin afectar mucho cuando se aplicaron despues. Un posible sistema de control para la maduración del ciclo reproductivo se deduce de estos resultados.

АБСТРАКТ

КОРРЕЛЯЦИЯ МЕЖДУ ИЗМЕНЕНИЯМИ НЕЙРОСЕКРЕТОРНОЙ
ДЕЯТЕЛЬНОСТИ И СОЗРЕВАНИЕМ ПОЛОВОЙ СИСТЕМЫ У
ARION ATER (STYLOMMATOPHORA, ARIONIDAE)

Б. Д. Смит

Изучалась нейросекреторная деятельность у слизняка *Arion ater* (L.) на различных стадиях цикла созревания половой системы этих моллюсков, для чего использовались серии срезов, окрашенных паральдегидфуксином.

Нейросекреция была обнаружена как в нервных клетках, так и в аксонах трех главных ганглиозных масс центральном нервной системы моллюсков (надглоточной, плевро-париетально-висцеральной и ножной), а также в буккальном ганглии в оптических тентакулах.

В начале созревания женских половых гонад наблюдается наибольшая корреляция между стадиями созревания половой системы и нейросекрецией. Только в это время наблюдается нейросекреторная активность некоторых мелких клеток париетально-висцерального ганглия. Эту стадию автор назвал "критической", поскольку искусственно созданные неблагоприятные условия, действующие до начала этой стадии сильно замедляют половое созревание, но почти не влияют на него после наступления этой стадии. Отсюда постулируется возможность контролирования стадий полового созревания у указанных.